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Expression of Bcl-2 and NF-κB in brain tissue after acute renal ischemia-reperfusion in rats

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ABSTRACT

Objective: To investigate the effect of acute renal ischemia reperfusion on brain tissue. **Methods:** Fourty eight rats were randomly divided into four groups (n=12): sham operation group, 30 min ischemia 60 min reperfusion group, 60 min ischemia 60 min reperfusion group, and 120 min ischemia 60 min reperfusion group. The brain tissues were taken after the experiment. TUNEL assay was used to detect the brain cell apoptosis, and western blot was used to detect the expression of apoptosis-related proteins and inflammatory factors. **Results:** Renal ischemia-reperfusion induced apoptosis of brain tissues, and the apoptosis increased with prolongation of ischemia time. The detection at the molecular level showed decreased Bcl-2 expression, increased Bax expression, upregulated expression of NF- κ B and its downstream factor COX-2/PGE2. **Conclusions:** Acute renal ischemia-reperfusion can cause brain tissue damage, manifested as induced brain tissues apoptosis and inflammation activation.

1. Introduction

Ischemia-reperfusion injury is a systemic pathological process. Renal ischemia-reperfusion injury is a relatively common tissue and organ damage in clinical practice, which can not only lead to changes in renal function but also cause severe multiple organ dysfunction syndrome. It can induce multiple organ dysfunction syndrome such as liver dysfunction, pancreatic trauma and brain tissue injury[1-3]. Among them the brain tissue is the most sensitive to renal ischemial-reperfusion injure and functional physiological and pathological changes.

Recently, more studies was performed to study the effect of distal ischemia-reperfusion and renal ischemia reperfusion on other tissues as well as the influence of renal ischemia reperfusion on brain tissue. Renal ischemia-reperfusion can cause brain damage, which has been confirmed by many researches. In this process, the effects of various neuregulins and signal transduction molecules have attracted more attention recently. However, the intracellular signal transduction pathways and biological function of brain damage caused by renal ischemia-reperfusion is still not completely clear, and whether the regulation of brain tissue cells apoptosis can cause the abnormal signal transduction of brain cells is still not clear[5-8]. Therefore, this study observed the changes of brain tissue apoptosis behavior, apoptosis signal verification and the expression of inflammatory factors, so as to investigate the effect of renal ischemic injury on brain tissue.

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2. Materials and methods

2.1. Experimental animals and grouping

A total of 48 adult male SD rats (260-300 g) rats, fasting 12 h preoperatively, were randomly divided into four groups (n=12): sham operation group (Group A), 30 min ischemia 60 min reperfusion group (Group B), 60 min ischemia 60 min reperfusion group (Group C), and 120 min ischemia 60 min reperfusion group (Group D).

2.2. Establishment of renal ischemia-reperfusion model

Rats were anesthetized with 10% (v/v) chloral hydrate by intraperitoneal injection and fixed. Abdomen was opened to make a 3-5 cm incision in the median laparatomy, and unilateral renal artery was separated. The kidney of successful reperfusion animal model turned from dark purple to red as soon as loosing the clamp. The brain tissue was obtained for successive test. The rats in the control group were received the same surgical treatment but without vascular clamping.

2.3. TUNEL assay

TUNEL (terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling) kits (Roche) were used for the detection of brain tissue apoptosis after acute renal ischemic. After fixation, the brain tissue was embedded, sectioned and detected in accordance with the instructions of TUNEL kit. The number of TUNEL-positive cells were detemined. Five fields of vision were chosen randomly to count the apoptotic cells, and the average value was taken as the apoptosis relative evaluation.

2.4. Western blot

The brain tissue was obtained from the rats in each group, and the protein was extracted by lysis method. After SDS-PAGE of 30 μg sample, the expression of apoptosis-related proteins Bcl-2/Bax and inflammatory cytokines NF-κB and COX-2 was detected by western blot method. β-actin was used as an internal reference.

2.5. ELISA

The brain tissue was added homogeneous buffer and centrifuged at 4 °C at 14 000 r/min for 10 min. The supernatant was used for determination of PGE_2 level according to the protocol of ELISA kit (Cayman Chemical, USA).

2.6. Statistical analysis

The data are expressed as mean±SD and one-way ANOVA was performed using SPSS/Win13.0 software (SPSS, Chicago, IL). P<0.05 was considered statistically significant.

3. Results

3.1. Acute renal ischemia-reperfusion induced brain cells apoptosis

Immunohistochemical examination showed that acute renal ischemia-reperfusion increased TUNEL-positive cells ratio in brain tissue. TUNEL-positive cells increased with ischemia time. Figure 1 shows only a small amount of apoptotic cells in the control brain tissue. The number of apoptotic cells in the brain tissue induced by reperfusion were increased 138.2% 203.9%, 365.7% in the groups A, B and C, respectively (P<0.05 *vs*. control).

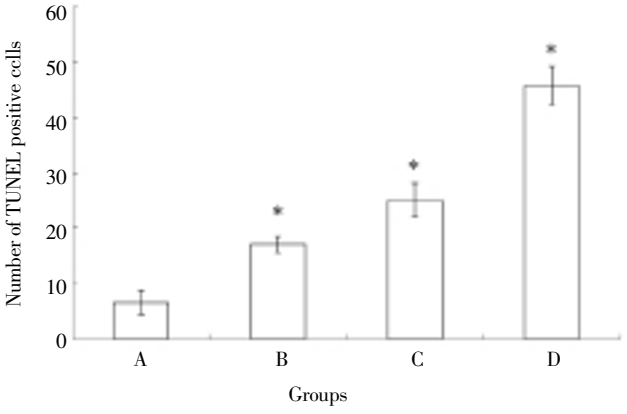

Figure 1. Number of apoptotic cells after renal ischemia-reperfusion. TUNEL assay were used for the detection of brain tissue apoptosis. Group A: sham operation group (Control); Group B: 30 min ischemia 60 min reperfusion group; Group C: 60 min ischemia 60 min reperfusion group; Group D: 120 min ischemia 60 min reperfusion group.
*P<0.05 *vs*. control.

3.2. Expression of apoptosis-related protein in brain cells

Western blot analysis showed that acute renal ischemia-reperfusion caused brain cell apoptosis, and the expression levels pf apoptosis-related proteins, Bcl-2/Bax, also changed. After acute renal ischemia-reperfusion, the Bcl-2 (inhibitor apoptosis protein) level in the brain tissue was decreased, while the Bax (pro-apoptotic protein) level was increased (Figure 2, P<0.05).

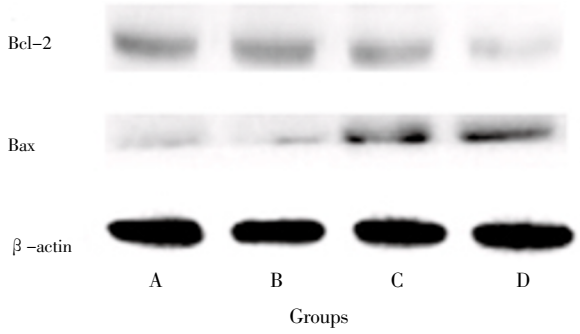

Figure 2. Effect of renal ischemia-reperfusion on expression of apoptosis-related proteins in brain tissue.
Group A: sham operation group (Control); Group B: 30 min ischemia 60 min reperfusion group; Group C: 60 min ischemia 60 min reperfusion group; Group D: 120 min ischemia 60 min reperfusion group. Renal ischemia-reperfusion reduced the Bcl-2 level in the brain tissue, and almost no Bcl-2 expression was observed in the 120 min ischemic group. Meanwhile, the apoptosis-inducing protein Bax expression increased significantly with the ischemic time.

3.3. Expression of inflammatory cytokines

The expression of NF-κB and COX-2 (Figure 3A) and PGE2 (Figure 3B) was detected by western and ELISA respectively. The results showed that the levels of NF-κB and its downstream factor COX-2 and PGE_2 in the brain tissue were significantly increased after acute renal ischemia reperfusion ($P<0.05$) and positively related with ischemic time.

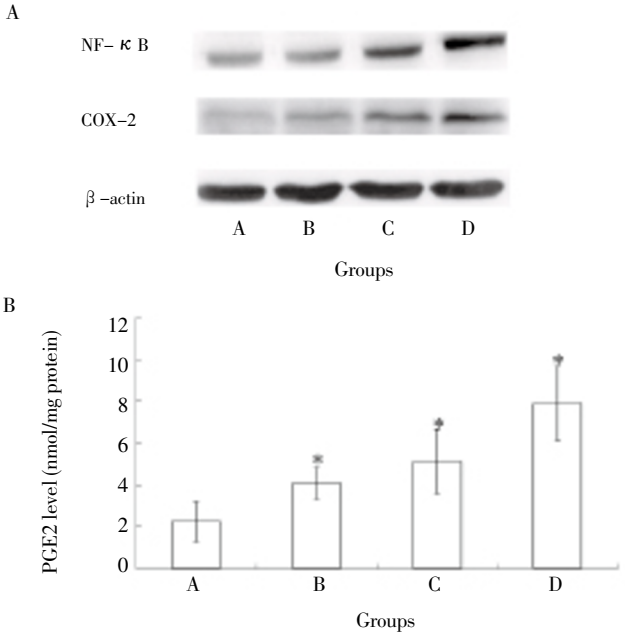

Figure 3. Effect of renal ischemia reperfusion on cytokine expression levels in brain tissue.
The expression of NF-κB and COX-2 was detected by western blot, while that of PGE_2 was also detected by ELISA kit. Group A: sham operation group (Control); Group B: 30 min ischemia 60 min reperfusion group; Group C: 60 min ischemia 60 min reperfusion group; Group D: 120 min ischemia 60 min reperfusion group. The expression of NF-κB, COX-2 and PGE_2 was significantly increased with the ischemic time after renal ischemia-reperfusion, while very low levels were observed in normal brain tissue.

4. Discussion

The ischemia-reperfusion injury is very complex. Studies have shown that post-treatment such as PI3K/Akt signal pathway, the mitochondria-sensitive potassium channel and extracellular signal-regulated kinase 1/2 (ERK1/2), can reduce ischemia-reperfusion injury[9-11]. However, the mechanisms of the effect of renal ischemia-reperfusion on brain tissue injury is still not very clear.

Bcl-2 gene is a proto-oncogene which can inhibit apoptosis. Bcl-2 family is currently divided into two categories: inhibit apoptosis such as Bcl-2 or promote apoptosis such as Bax. Research has shown that Bax expression was significantly increased in traumatic hemorrhagic shock rats, and the Bax expression is closely related to cell apoptosis of the tissue. In this study, we found that the Bax expressions of brain tissue were significantly increased after renal ischemia-reperfusion, which indicated the cells apoptosis of brain tissue. Meanwhile, the Bcl-2 expression decreased, indicating that renal ischemia-reperfusion had an impact on Bcl-2 family apoptosis-related proteins.

NF-κB is an important transcription factor which can regulate a variety of cytokines and protein gene transcription. Numerous studies have demonstrated that inflammation has an important role in ischemia-reperfusion injury. Under normal conditions, NF-κB activation and inactivation are in a dynamic equilibrium state, and most of the NF-κB is located in the cytoplasm and in an inactivated state. When cells are influenced by outside stimulation (such as reactive oxygen species, abnormal signal transduction, ischemia, and acute stress), NF-κB in the inactivated state can be activated rapidly and transferred to the nucleus and specifically binds to promoter or enhancer sites of the target gene, thereby initiating the transcription expression and regulating target genes. In addition to regulation of downstream inflammatory factors such as COX-2/PGE_2 and inflammation, NF-κB can also participate in a variety of biological processes including apoptosis. In this study, we found that the inflammation NF-κB pathway of brain tissue was activated after renal ischemia-reperfusion, and the expression was also raised with ischemic time. The downstream COX-2/PGE_2 expression is also significantly increased[15-19].

Although the *Bcl-2* gene promoter also has sites which can bind NF-κB, NF-κB can be activated by the induction of *Bcl-2* and other anti-apoptotic genes to prevent apoptosis, and the signal which can regulate apoptosis significantly enhanced with the ischemia-reperfusion period. For

example, stimulating *c-myc* and *TNF-α* gene expression can lead to apoptosis. The mechanisms of ischemia-reperfusion induced brain cell apoptosis need further study[19-21].

In conclusion, this study confirmed the renal ischemia-reperfusion can induce apoptosis of brain tissue, may be due to the regulation by the Bcl-2/Bax apoptosis protein. Inflammatory signal NF-κB may be involved in renal ischemia reperfusion injury and other organs damage including brain, and the specific mode of action and mechanisms need to be further studied.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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